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Effect of dietary protein level on retention of nutrients, growth performance, litter composition and NH_3 emission using a multi-phase feeding programme in broilers

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Abstract

Three experiments were conducted to study the effect of dietary protein level on the retention of nutrients, growth performance, litter composition and NH_3 emission in broiler chickens kept under laboratory conditions (housed in cages, Exp.1) or in commercial conditions (in pens, Exp.2; or whole houses of a farm, Exp.3). All the trials were performed according to a factorial experimental design, involving a 4-stage feeding programme and two levels of dietary crude protein (CP) for each period: control *vs*. low crude protein (CP reduced by 1.5%). In Exp.1, the coefficients of total tract apparent retention of dry matter and CP were higher in the birds fed the low CP diet ($p < 0.05$). On average, reducing the CP of the diet led to a 4.8% reduction in the nitrogen excreted per CP intake. In Exp.2, the feed conversion ratio was higher in birds fed the low CP diet from 22 to 35d ($p < 0.05$), from 35 to 42d ($p < 0.01$), and over the whole experimental period ($p < 0.01$). In Exp.3, low CP diets decreased the nitrogen content of the litter in the finisher period ($p < 0.05$). The average NH_3 concentration and emission from 33 to 42d were lower in the low CP house ($p < 0.01$), with a 16% decrease in the cumulative NH_3 emission. Therefore, the reduction in dietary CP content by 1.5% reduced the potential environmental impact, although it had a negative effect on the feed efficiency of broilers.

Additional key words: broiler performance; nitrogen excretion; dietary protein; multi-phase feeding programme.

Introduction

Environmental concerns associated with livestock production have increased in recent years and EU countries are increasingly focusing on extensive and restrictive policies. The European IPPC Directive 1996/61/EC on Integrated Pollution Prevention and Control, updated by Directive 2010/75/EU (OJ, 2010), aims to achieve integrated systems for the prevention of polluting emissions from industrial sectors, in which the intensive rearing of poultry is included. For each of the categories of industrial activities listed in the Directive, there are reference documents indicating the application of best available techniques (BAT) in order to prevent, or reduce, emissions to air, land and water. The first reference document for intensive livestock farming concerned the intensive rearing of poultry and pigs. These documents have been adapted by some countries to the specific features of each livestock sector (production systems, climate conditions, etc.).

One of the main goals of BAT is a reduction in NH_3 emissions. In Spain, guidelines for the application of BAT to the rearing of broilers involve nutritional techniques, improvements in the design of facilities and manure management practices (MARM, 2010). Among nutritional techniques, the guide recommends adaptating the levels of protein to the requirements of birds according to the different periods of production, and formulation of feeds based on amino acid (AA) levels suitable for optimal development, thus limiting their excess and environmental accumulation. There is evidence that dietary manipulation, in order to provide optimal essential AAs in the diet in non-ruminant animals, could contribute to the more efficient use of

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Abbreviations used: AA (amino acid); ADFI (average daily feed intake); ADG (average daily gain); BAT (best available techniques); BW (body weight); CIAD (coefficients of ileal apparent digestibility); CP (crude protein); CTTAR (coefficients of total tract apparent retention); DM (dry matter); FCR (feed conversion ratio); N (nitrogen).

these nutrients. Any AAs surplus, above the birds' requirements, is deaminated and nitrogen (N) is excreted as uric acid (Kamran *et al*., 2004).

A decrease on dietary protein levels applied to poultry production showed that N excretion or NH_3 emission could be reduced (Jacob *et al*., 1994; Elwinger & Svensson, 1996; Ferguson *et al*., 1998a; Khajali & Moghaddam, 2006). Although most research has been conducted with birds housed in cages or pens, very few studies have been developed under real commercial conditions (*e.g.,* Robertson *et al*., 2002). Furthermore, most studies on reducing dietary protein have been carried out with feeding programmes involving a small number of diets: from 1 to 21d and from 22 to 42d.

Some studies have shown that low protein diets in broilers can lead to poorer performance or feed efficiency, despite supplementation with essential AAs (Blair *et al*., 1999; Bregendahl *et al*., 2002); although other studies found no such effects (Han *et al*., 1992; Hai & Blaha, 1998). The variability and interpretation of results reported in the literature could be partly explained by factors such as age, sex, genotype and management strategies (Emmert & Baker, 1997; Samadi & Liebert, 2006; Tolomir *et al*., 2010).

The objective of this study was to determine the effect of a reduction in dietary protein, using a multiphase feeding programme in broilers fed wheat-based diets supplemented with AAs, on nutrient retention, growth performance, litter composition and NH_3 emission under European commercial conditions.

Material and methods

General feeding programme

All trials were performed according to a factorial experimental design, involving a 4-stage feeding programme —starter (1 to 10d), grower (11 to 21d), finisher (22 to 35d) and withdrawal (36 to 42d) diets—, and two levels of dietary crude protein (CP) for each period (control *vs.* low CP).

For each feeding stage, two levels of dietary CP were formulated: a control diet (with protein levels of 23.5, 22.5, 21.5 and 20.5%, respectively), and a low CP diet (with 1.5% less CP per period). The diets were formulated to meet or exceed the requirements concerning faecal digestible lysine, methionine, threonine and tryptophan recommended by FEDNA (2008), and to be isocaloric within each period. All diets for each period were prepared with the same batch of ingredients. The ingredient composition and nutrient content of the diets are shown in Table 1. The starter and grower feeds were offered in crumbles, and the finisher and withdrawal diets were steam pelleted using a 4 mm die. All diets included 5 g titanium dioxide kg^{-1} (Coralim, Valencia, Spain) as an indigestible marker. Water and feed were provided *ad libitum*.

During the course of this study, broilers were handled according to the Spanish legislation concerning the protection of animals used for experimental and other scientific purposes (BOE, 2007).

Experiment 1

An experiment with 200 one-day-old Ross 308 male broilers was conducted from 1 to 42d in an environmentally controlled room. At one day old, birds were allotted to 8 wire cages (70 cm × 60 cm) of 25 chicks each. Each cage represented one replicate (4 replicates per treatment). The cages were equipped with two water cups, two feeding troughs and an excreta collection tray placed below the cages. The temperature was gradually reduced from 32°C during the first 5 days to 20°C on day 40. The lighting cycle was 24 h d^{-1} from 1 to 3d, 18 h d^{-1} from 4 to 39d, and 23 h d^{-1} from 40 to 42d.

The water intake per cage was determined daily by volumetric difference between water supplied and water refused. To calculate the coefficients of total tract apparent retention of nutrients (CTTAR), excreta from each cage were collected daily from 6 to 9d, from 17 to 20d, from 28 to 31d and from 38 to 41d and mixed for each period. Then, a representative sub-sample of homogenized fresh excreta was taken for each cage and kept frozen (–20°C) until analysis. The coefficients of ileal apparent digestibility of nutrients (CIAD) were determined on days 10, 21, 28 and 42 from 10, 7, 3 and 3 birds per replicate, respectively. All birds were randomly selected, weighed and then killed by cervical dislocation. The digestive tract was immediately exposed, and the contents of the ileum (from Meckel's diverticulum to 40 mm above the ileo-cecal junction) were collected by gently flushing with distilled water into plastic containers. The ileal digesta of all the birds within a replicate were pooled, lyophilized, ground through a 0.5 mm sieve and stored in airtight containers at –20°C until analysis. The body weight (BW) of the broilers slaughtered at 42d and the empty BW (BW

Table 1. Ingredients and composition of experimental diets (as-fed basis)

Item	Starter (1-10d)		Grower (11-21d)		Finisher (22-35d)		Withdrawal (36-42d)	
	Control	Low CP	Control	Low CP	Control	Low CP	Control	Low CP
Ingredients, g kg^{-1}								
Soybean meal (470 g CP kg^{-1})	292	246	273	226	253	204	242	193
Sorghum	150	150	150	150	200	200	150	150
Wheat	178	227	306	359	336	393	357	413
Maize	200	200	100	100	40	40	80	80
Dried bakery by-product	60	60	50	50	50	50	60	60
Soy oil	24.7	16.7	26.6	26.9	10.5	13.1	5.7	8.4
Animal fat	—	—	10.8	—	37.6	23.0	43.1	28.5
Fish meal (620 g CP kg^{-1})	60	60	50	50	40	40	30	30
Calcium carbonate	8.5	8.7	9.0	9.2	9.3	9.4	9.6	9.7
Monocalcium phosphate	4.8	5.2	4.8	5.3	5.1	5.4	5.3	5.5
Titanium dioxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salt	1.6	0.4	1.3	0.1	1.4	0.1	1.8	0.4
L-Lysine (500 g kg^{-1})	3.6	5.8	3.1	5.5	2.8	5.1	2.0	4.3
DL-Methionine (990 g kg^{-1})	3.3	3.7	3.1	3.4	2.8	3.2	2.5	2.8
L-Threonine (980 g kg^{-1})	0.6	1.2	0.4	1.1	0.3	0.9	0.2	0.8
Sodium bicarbonate	2.9	5.2	0.3	3.5	1.2	2.8	0.8	2.6
Vitamin and mineral premix[a,b]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Calculated composition[c]								
Metabolizable energy, MJ kg^{-1}	12.5	12.5	12.9	12.9	13.1	13.1	13.2	12.2
Crude protein (CP), g kg^{-1}	235	220	225	210	215	200	205	190
Ileal digestible amino acid, g kg^{-1}								
Lysine	13.0	13.0	12.0	12.0	11.0	11.0	11.1	11.1
Methionine + cysteine	9.6	9.6	9.1	9.1	8.6	8.6	8.9	8.9
Threonine	8.0	8.0	7.4	7.4	7. 0	7.0	7.4	7.4
Tryptophan	2.4	2.2	2.4	2.2	2.3	2.1	2.5	2.2
Calcium	8.4	8.4	8.0	8.0	7.6	7.6	7.2	7.2
Total phosphorus	6.4	6.3	6.1	6.0	5.8	5.7	5.5	5.4
Available phosphorus	4.3	4.3	4.1	4.1	3.9	3.9	3.7	3.7
Analysed composition[d], g kg^{-1}								
Dry matter	893	896	894	898	908	902	894	891
CP	232	218	225	209	214	198	206	190

[a] Supplied per kg of starter and grower diets: vitamin A (retinyl acetate), 11,000 IU; vitamin D3, 5,000 IU; vitamin E (DL-α-tocopheryl acetate), 80 mg; vitamin K, 3 mg; thiamin, 3 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vitamin B_{12}, 0.02 mg; niacin, 70 mg; folic acid, 2 mg; biotin, 0.15 mg; choline, 500 mg; pantothenic acid, 15 mg; Mn, 120 mg; Zn, 80 mg; Fe, 50 mg; Cu, 10 mg; I, 1.1 mg; Se, 0.3 mg; Co, 0.05 mg. Natugrain (endo-1,4-β-xylanase), BASF Group, Ludwigshafen, Germany. Natuphos (3-phytase), BASF Group, Ludwigshafen, Germany. [b] Supplied per kg of finisher and withdrawal diets: vitamin A (retinyl acetate), 9,000 IU; vitamin D_3, 30,00 IU; vitamin E (DL-α-tocopheryl acetate), 48 mg; vitamin K, 2.5 mg; thiamin, 1.8 mg; riboflavin, 6 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.012 mg; niacin, 40 mg; folic acid, 1 mg; biotin, 0.1 mg; choline, 400 mg; pantothenic acid, 12 mg; Mn, 100 mg; Zn, 70 mg; Fe, 30 mg; Cu, 8 mg; I, 1 mg; Se, 0.3 mg; Co, 0.05 mg. Natugrain (endo-1,4-β-xylanase), BASF Group, Ludwigshafen, Germany. Natuphos (3-phytase), BASF Group, Ludwigshafen, Germany. [c] According to FEDNA (2010). [d] Samples in triplicate.

without the digestive tract and its contents) were individually recorded. The empty BW was expressed relative to the BW. In addition, the weights of left breast and left thigh were also recorded and adjusted for the empty BW.

Experiment 2

A total of 84 one-day-old Ross 308 male chickens were randomly divided into groups of 7 birds and housed in 12 floor pens (1 m × 1 m), with six pens per treat-

ment. Each pen represented one replicate. The pens were situated on a commercial farm and the animals were reared with their contemporaries of the same flock from 1 to 42d. Each pen was equipped with a feeding trough and four nipple drinkers, and covered with wood shavings. The temperature and lighting programmes were identical to those described in Experiment 1.

Birds were weighed on days 1, 10, 21, 35 and 42, and feed intake per pen was recorded for each period. Mortality was checked daily and the weight of dead chickens was also recorded at the time of removal. The average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were calculated on a pen basis from these data.

Experiment 3

This experiment was carried out with fifty-six thousand one-day-old Ross 308 broiler chickens of both sexes (50/50) in a commercial farm. Broilers were placed in two twin poultry houses (28,000 birds per house). One poultry house was used as control treatment, and the birds in the other house were fed the low CP diet. The rearing period of the broilers lasted 42d. Each bird had an area of approximately 0.09 m^2. Each poultry house was equipped with four feeding troughs and five nipple drinker lines, whose floor was covered with clean wood shavings. The ventilation system consisted of nine fans located in the end-walls of each poultry house and automatically adjusted according to the inside/outside temperature, and an evaporative cooling system located in the sidewalls of the house. Temperature and lighting programmes were identical to those described in Experiment 1.

Litter was sampled at the end of each period (on days 10, 21, 35 and 42) from each poultry house. To sample the litter in each house, they were divided transversely into two halves, taking two litter samples per house. Each sample corresponded to sub-samples taken from 10 random places in a zigzag pattern, and they were obtained from the full depth of the litter (away from the feeders and drinkers) using a 17 cm × 12 cm rectangular plastic cage. The random litter sub-samples were thoroughly mixed and homogeneized, and 250 g was weighed and delivered to the laboratory for further processing. A fraction of each sample was immediately dried at 80°C for 48 h, while the rest of the sample was ground to pass through a 2 mm sieve, and frozen at –20°C in airtight containers until further analysis.

The concentration and emission of NH_3 from the poultry houses were continuously monitored during the last 10 days of the flock-rearing period, when the animals were 33 to 42 days old. To estimate NH_3 emission rates from the facilities, it was necessary to measure both the ventilation rate and gas concentration. The ventilation flow was indirectly determined by a balance method based on indoor and outdoor CO_2 concentrations (CIGR, 2002). Gas concentrations (CO_2 and NH_3) were continuously monitored by X-am® 7000 Dragër multigas detector (Dräger, Lübeck, Germany). The detectors, one device per house, were placed inside at random locations (1 m above floor) and close to the ventilation system exhaust outlet, while another one was located outside. Using infrared technology, the CO_2 sensor could detect it in the range 0-90,000 mg m^{-3}, with a resolution of 180 mg m^{-3} (Dräger Sensor® Smart IR CO_2), while a catalytic sensor could measure NH_3 in the range 0-139 mg m^{-3}, with a resolution of 0.7 mg m^{-3} (Dräger Sensor® XS NH_3). Both sensors were calibrated at the beginning of the study by the suppliers. Data (gas concentrations) were recorded continuously throughout this period, and the 10-min averages were stored by a data logging system for later analysis.

Analytical procedure

The dry matter (DM) content of feeds was determined by oven-drying at 103°C for 8 h until constant weight, and the same process being followed for excreta and litter but at 80°C. Feeds, ileal digesta, excreta and litter samples were analysed for N by the Kjedhal technique (AOAC, 1990). The CP content was estimated by multiplying the N content by 6.25. The titanium dioxide content of feed and ileal digesta was analysed by the procedure described by Myers *et al*. (2004) and the CIAD coefficients of CP were calculated from these data. The pH and electrical conductivity of the litter were evaluated in a 1:2 ratio of fresh litter to distilled water as described by Peters *et al*. (2003).

Statistical analysis

All data were analyzed as a completely randomized design, with a factorial arrangement (4 × 2) and using the ANOVA procedure of the SPSS software (SPSS Inc., Chicago, IL, USA). The model included the effect

of feeding stage and level of dietary CP as main effects, and the first order interaction effect. Dietary treatment comparisons by feeding period were performed by using F-test. Concentration of NH_3 and emission data were compared using Students' t test for unpaired data. The significance level was set at $p < 0.05$, with $0.05 < p < 0.10$ considered a trend.

Results

Experiment 1

Table 2 shows the effect of dietary treatments on CTTAR, CIAD, N excretion, DM of faeces and daily water intake of the broilers. There were differences between dietary treatments as regards the CTTAR of DM and CP ($p < 0.05$). On average, the CTTAR of DM and CP of birds fed the control diet were lower than those found in birds fed the low CP diet. In addition, the N content of faeces and N excretion relative to both CP and DM intake were higher when birds were fed the control diet ($p < 0.05$). However, no effect of the diet on the CIAD of DM and CP were observed ($p > 0.05$). As broilers aged from 1 to 42d, both the CTTAR and CIAD of DM and CP decreased ($p < 0.001$), and N excretion increased ($p < 0.001$). No interaction between dietary treatment and feeding stage was found on these variables ($p > 0.05$).

The DM of faeces and daily water intake of the broilers were affected by dietary treatment. The DM of faeces was lower in the birds fed the control diet than in birds fed the low CP diet ($p < 0.05$). The higher faeces moisture content was accompanied by a higher daily water intake in the control diet ($p < 0.01$), although there was an interaction tendency between dietary treatment and feeding period in both variables ($p = 0.087$ and $p = 0.063$ for DM of faeces and water intake, respectively).

Table 3 presents the effect of treatments on BW and empty BW, breast and thigh yield of broiler at 42d. No effects were observed ($p > 0.05$).

Experiment 2

Table 4 presents the effect reduced dietary CP on the productive performance recorded for each feeding phase and for the whole period. No effects of dietary treatments on ADG and ADFI were observed for the

Table 2. Effect of dietary crude protein (CP) on coefficients of total tract apparent retention (CTTAR) and ileal apparent digestibility (CIAD), nitrogen excretion, dry matter (DM) of faeces and water intake (Exp. 1)

	Starter (1-10d)		Grower (11-21d)		Finisher (22-35d)		Withdrawal (36-42d)		SEM[a]	Source of variation		
	Control	Low CP	Control	Low CP	Control	Low CP	Control	Low CP		Diet	Age	Diet × Age
CTTAR												
DM	0.76	0.77	0.71^{x}	0.74^{y}	0.71	0.72	0.70	0.71	0.002	0.009	< 0.001	0.526
CP	0.67	0.67	0.58^{x}	0.62^{y}	0.59	0.58	0.49	0.52	0.004	0.042	< 0.001	0.238
CIAD												
DM	0.83	0.83	0.66	0.67	0.68	0.68	0.68	0.64	0.004	0.648	< 0.001	0.279
CP	0.88	0.87	0.72	0.73	0.78	0.77	0.61^{y}	0.55^{x}	0.005	0.138	< 0.001	0.262
Nitrogen excretion												
N faeces, g kg^{-1} DM	56.2^{y}	54.1^{x}	58.0^{y}	53.8^{x}	53.7^{y}	51.1^{x}	57.0^{y}	52.3^{x}	0.22	< 0.001	< 0.001	0.137
N excretion, g kg^{-1} CP intake	52.8	52.3	67.6^{y}	60.3^{x}	66.1	66.4	82.2	76.9	0.75	0.042	< 0.001	0.239
N excretion, g kg^{-1} DM intake	13.2	12.4	16.7^{y}	14.0^{x}	15.3	14.1	17.3^{y}	15.0^{x}	0.17	< 0.001	< 0.001	0.185
DM of faeces, g kg^{-1}	247.4	239.7	210.5	219.8	209.3^{x}	235.0^{y}	188.7	198.6	2.18	0.044	< 0.001	0.087
Water intake, mL $bird^{-1}$ d^{-1}	52.3	52.6	149.6	137.6	356.5	318.3	660.3^{y}	581.5^{x}	5.23	0.005	< 0.001	0.063

Means within a row in each period with different superscripts $^{(x\text{-}y)}$ are significantly different ($p < 0.05$). Contrasts by period were performed by using F-test. [a] Standard error of the mean. n = 4 replicates per treatment.

Table 3. Effect of dietary crude protein (CP) on body weight (BW) and empty BW, breast and thigh yield of broilers at 42d (Exp. 1)

	Diets		SEM[a]	*p*-value
	Control	Low CP		
BW, g	2,970	2,889	27.7	0.155
Empty BW, g kg^{-1} BW	822	821	2.9	0.781
Left breast, g kg^{-1} empty BW	110	106	1.4	0.121
Left thigh, g kg^{-1} empty BW	116	115	0.8	0.661

[a] Standard error of the mean. n = 4 replicates per treatment.

whole experimental period (from 1 to 42d). However, a reduction in dietary CP tended to decrease ADG for the starter and grower periods compared with broilers fed the control diet ($p = 0.098$ and $p = 0.061$, respectively). In addition, ADFI (from 1 to 10d) and BW at 21d were higher in birds fed the control diet ($p < 0.05$). In contrast, from 35 to 42d, ADFI in birds fed the low CP diet was higher than that found in birds fed the

Table 4. Effect of dietary crude protein (CP) on body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broiler chickens from 1 to 42d (Exp. 2)

	Diets		SEM[a]	*p*-value
	Control	Low CP		
Starter (1-10d)				
BW, g	231	215	4.7	0.098
ADG, g	21.5	19.8	0.51	0.098
ADFI, g	23.8	20.8	0.73	0.035
FCR	1.10	1.06	0.010	0.130
Grower (11-21d)				
BW, g	799	731	17.4	0.045
ADG, g	51.6	46.8	1.29	0.061
ADFI, g	67.3	63.2	1.35	0.135
FCR	1.30	1.35	0.021	0.333
Finisher (22-35d)				
BW, g	1,990	1,909	29.2	0.176
ADG, g	85.1	84.1	1.36	0.746
ADFI, g	132.7	134.9	1.73	0.548
FCR	1.56	1.63	0.018	0.044
Withdrawal (36-42d)				
BW, g	2,721	2,622	32.0	0.128
ADG, g	104.4	100.4	2.20	0.391
ADFI, g	177.8	192.2	2.92	0.001
FCR	1.71	2.00	0.053	0.001
Whole period (1-42d)				
BW, g	—	—	—	—
ADG, g	65.5	63.1	0.78	0.128
ADFI, g	98.9	101.6	1.19	0.295
FCR	1.51	1.61	0.018	0.001

[a] Standard error of the mean. n = 6 replicates per treatment.

control diet ($p < 0.01$), but no effect on ADG was observed ($p > 0.05$). As regards feed efficiency, FCR was higher in birds fed the low protein treatment from 22 to 35d ($p < 0.05$), from 35 to 42d ($p < 0.01$), and over the whole experimental period ($p < 0.01$).

Experiment 3

The effect of reduced dietary CP levels on litter characteristics at 42d is shown in Fig. 1. There was no effect on moisture and pH of the litter ($p > 0.05$). However, the N content in the litter was affected by the dietary treatment ($p < 0.05$), although there was also an interaction between the level of dietary CP and feeding period ($p < 0.05$). The CP level affected the N of the litter for the finisher diet ($p < 0.05$), the N content being lower when birds were fed the low CP diet. Moreover, the reduction in dietary CP tended to reduce the electrical conductivity of the litter ($p = 0.075$). All the studied litter variables were affected by feeding period ($p < 0.001$), values increasing with time ($p < 0.001$).

Fig. 2 presents the effect of dietary treatments on the concentration and cumulative emission of NH_3 from the two commercial poultry houses during the last period of the experiment, when the animals were 33 to 42 days old. The average NH_3 concentration was higher in the control house than that in the low CP house (5.37 *vs.* 4.14 mg · m^{-3}, respectively; $p < 0.001$). The estimated NH_3 emissions from 33 to 42d were also higher in the control house (daily average of 7.92 *vs.* 6.65 mg NH_3 per bird and hour, respectively; $p < 0.01$). As a result, the cumulative NH_3 emission from the control house was higher than that of the facility housing birds fed the low CP diet (1,901 *vs.* 1,596 mg NH_3 per bird and per period, respectively)

Discussion

Experiment 1

In the current study, low CP diets supplemented with synthetic AAs did not affect CIAD of DM and CP.

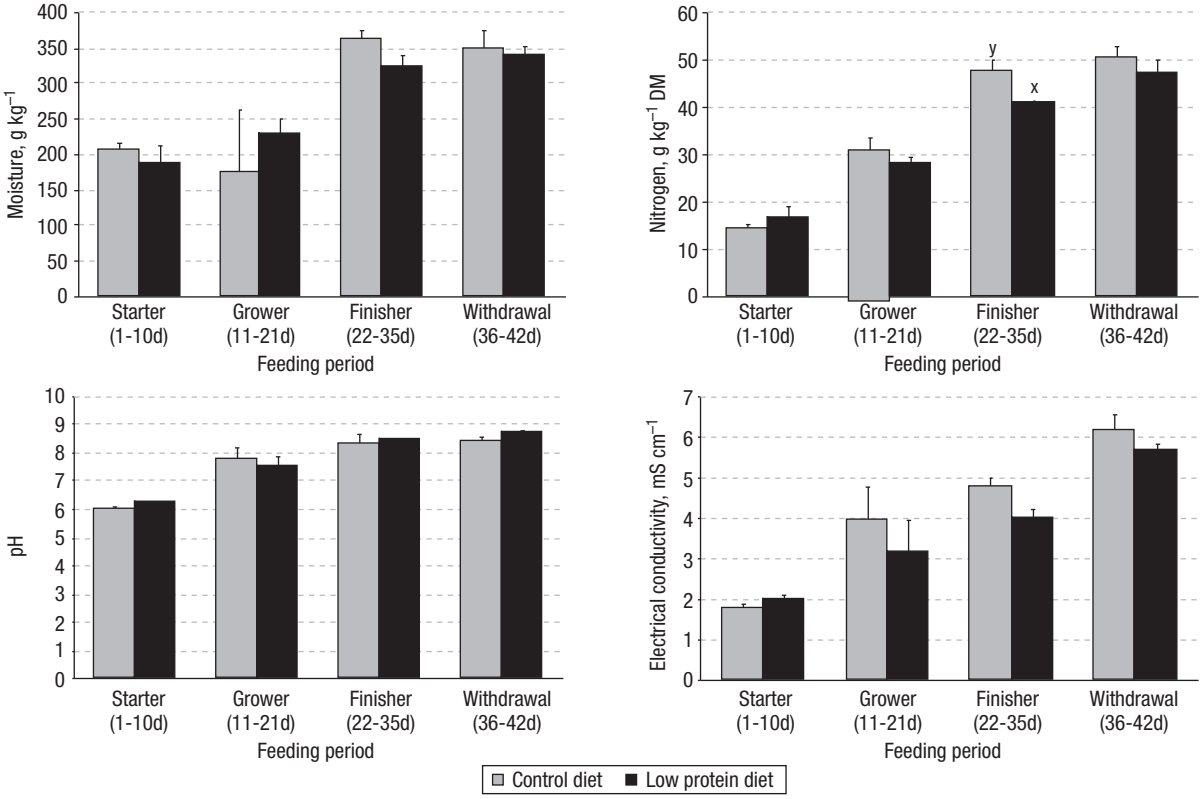

Figure 1. Effect of dietary crude protein on litter characteristics at 42d (Exp. 3). Means within period with different superscripts (x-y) are significantly different ($p < 0.05$). Contrasts by period were performed by using F-test. Data are the mean ±standard deviatio. n = 2 replicates per treatment.

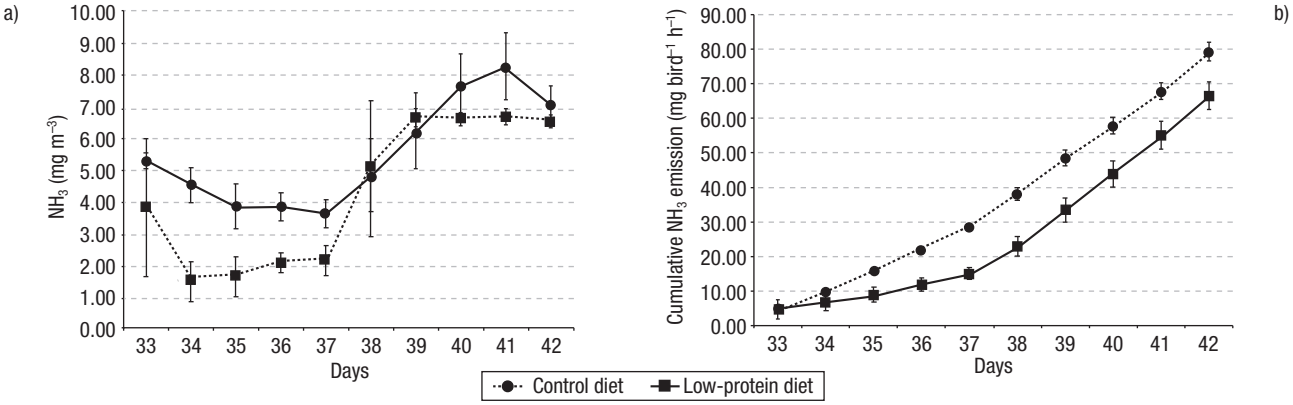

Figure 2. Effect of dietary crude protein on ammonia concentration (a) and cumulative NH_3 emission (b) from 33 to 42d in twin poultry houses (Exp. 3). Data are the mean ± standard deviation. n = 24 data per treatment.

However, CTTAR was lower in birds fed the control diet, both the average N content of faeces and N excretion relative to CP intake or to DM intake decreasing. On average, a 4.8% reduction in N excreted per CP intake was obtained when the CP of the diet was decreased 1.5%. Blair *et al*. (1999) reported a similar reduction in N excreted per CP intake (10.1% for a reduction in dietary CP of 3%). Yamazaki *et al*. (1998) indicated that N retention was increased by the use of low CP diets supplemented with AAs; although, the N excreted (expressed as a percentage of N intake) did not decrease in diets supplemented with an excess of AAs (Yamazaki *et al*., 2006). In this sense, Kerr & Kidd (1999) indicated that N excretion decreased following a moderate reduction in the CP content of the diet supplemented with AAs, but not when CP was strongly reduced. Therefore, N utilization depends on the proper supply of AAs, avoiding an excess or deficit of a given essential AA or, even, an imbalanced ratio of essential to non-essential AAs. Such imbalances could alter metabolic protein utilization by increasing AA catabolism and excretion of N as uric acid.

Our results concerning the reduction of N excretion agree with those of other studies conducted with birds fed maize-based (Kerr & Kidd, 1999) or wheat-based (Blair *et al*., 1999) diets. Differences between cereal grains in terms of protein content and quality should be taken into account, since this could affect N utilization.

As expected, the reduction in dietary CP affected the DM content of faeces and the daily water intake of the broilers. The reduction in water consumption by lowering the dietary CP content is a well-known effect (Bailey, 1999), while dietary changes leading to lower water intake are expected to reduce excreta and litter moisture. Our results showed that the DM of faeces was lower in birds fed the control diet than in birds fed the low CP diet. In additon, the higher moisture content of faeces was accompanied by higher daily water intake. A higher protein intake increases the amount of metabolites eliminated in urine, and the water is necessary to excrete nitrogenous waste products from the protein metabolism. Broilers fed low CP diets reduced their water intake, independently of environmental temperature (Alleman & Leclerq, 1997) and sex (Hernández *et al*., 2012). Moreover, Elwinger & Svensson (1996) found that protein level influenced not only the amount of water used, but also the water:feed ratio.

The levels of CP did not affect on empty BW, breast and thigh yield of broilers at 42d, probably because of the balance and proper supply of most limiting essential AAs in low CP diets (Si *et al*., 2001; Baker *et al*., 2002). Similar results on carcass yield were found by Khajali & Moghaddam (2006), in an experiment to determine the effect of low CP diet supplemented with DL-methionine; or by Kamran *et al*. (2008), who did not observe differences in carcass yield and abdominal fat in broilers fed low CP diets with a constant ME:CP ratio. Deschepper & De Groote (1995) did not find differences in carcass yield, using an ideal AA balance, although low CP diets resulted in a higher carcass fat content.

Experiment 2

The growth performance experiment showed that broilers fed low the CP diet had lower BW than those fed control diet at 21d. Hussein *et al*. (2001) indicated that supplementation with AAs and extra energy can

only partially correct the depression in growth performance observed with low CP diets. In addition, low CP diets (Kerr & Kidd, 1999) and defficiencies of some essential AAs (Moran, 1994) could reduce weight gain and feed efficiency. However, in the current experiment, no effects on FCR were found for the first 21 days. In contrast, ADFI (from 35 to 42d) and FCR (from 22d onwards and over the whole experimental period) were higher in birds fed low the CP diet than in birds fed the control diet. Others authors have also reported a significant increase in ADFI and FCR in broilers fed low CP diets supplemented with AAs (Ferguson *et al*., 1998a; Bregendahl *et al*., 2002), although some studies found no effect on ADFI or FCR (Han *et al*., 1992; Hai & Blaha, 1998). The discrepancies observed among authors could be due to many factors, including the relationships between dietary CP levels, energy levels, and AA supplementation, or factors influencing the birds' requirements, such as age, sex, genotype, and management strategies.

Experiment 3

No effects on the moisture content or pH of the litter were detected for the dietary CP content, in agreement with previous studies (Elwinger & Svensson, 1996). In contrast, Ferguson *et al*. (1998a) reported an increase in litter acidity in the low CP diet compared with a control diet, perhaps due to the drier litter. This could help reduce NH_3 production by inhibiting the microorganisms which hydrolyze uric acid. In addition, the lower pH reduces the non-ionized form (NH_3 to NH_4^+) of ammonia, decreasing its volatilization (Ferguson *et al*., 1998b; Khajali & Moghaddam, 2006). On average, the N content of the litter was 7.4% lower for low CP diets (with 1.5% less CP). In a study by Ferguson *et al*. (1998b), the N content of the litter was decreased by 7% for each percentage unit reduction in dietary CP. In general, lower dietary CP levels have been seen to lead to a reduction in the N content of the litter (Jacob *et al*., 1994; Elwinger & Svensson, 1996; Fergusson *et al*., 1998b; Khajali & Moghaddam, 2006). However, these studies were carried out with feeding programmes involving a reduced number of diets, usually two. Our results confirm that, even in a multi-phase feeding programme, the excretion of N was decreased with reduced levels of dietary CP.

The pollutant capacity of the litter increases with the age of the birds and NH_3 emissions are expected to be particularly noticeable during the last days of the flock period (Amon *et al*., 1997). The NH_3 concentrations and emmissions were higher in the control house compared to those found in the low CP house. Also, in commercial-scale studies, Robertson *et al*. (2002) showed a correspondence between NH_3 emission and the total protein intake. Using a dynamic chamber technique and passive diffusion samplers in individual pens, Elwinger & Svensson (1996) identified linear trends of increasing NH_3 emission rates with higher diet CP contents.

In the current trial, there was a 16% decrease in cumulative NH_3 emission linked to the reduction in dietary CP levels. However, the estimated averages in both houses were lower than those estimated for birds raised on reused litter in multiple flocks (Lacey *et al*.. 2003). Under commercial conditions in Europe, where litter is renewed between flock cycles, Calvet *et al*. (2011) reported lower average NH_3 emission rates than those predicted by Lacey *et al*. (2003), even though they were higher than our estimates. Despite the use of fresh litter and the lower emission values obtained, the current study showed that NH_3 emissions decreased as the level of dietary CP was reduced.

In conclusion, the reduction of 1.5% per period in the CP content of the diet in a multi-phase feeding programme in male broilers fed wheat-based diets decreased N excreted by 4.8% (as measurement relative to the CP intake). From a productive point of view, low levels of CP did not affect carcass yield, but FCR was impaired. Under European commercial conditions of broiler production, a multi-phase feeding programme with low CP content in the diet could be used to reduce the N content of the litter, and the concentration and emission of NH_3 from broiler facilities.

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